DAIDS

VIROLOGY MANUAL

FOR HIV LABORATORIES

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Compiled by

THE DIVISION OF AIDS

NATIONAL INSTITUTE OF ALLERGY & INFECTIOUS DISEASES

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and

COLLABORATING INVESTIGATORS

PIPETTE CALIBRATION PROCEDURES

I. PRINCIPLE

Using accurate pipettes is one of the key factors in obtaining good, reliable assay results. With time and use, pipettes become worn and less reliable. Thus, pipettes should be maintained regularly to ensure their accuracy as well as increase their longevity. Each pipette should be calibrated when it is first received and quarterly thereafter to preserve pipette accuracy. Expiration dates for the next calibration should be attached to the pipette. The precision of a pipette may be determined by making several measurements at selected settings of the pipette and calculating the coefficient of variation of those values.

One method for measuring accuracy is carried out by weighing the volume of water delivered by a pipette at selected settings and then calculating the average weight/volume. The calculated weight/volume is then compared to the theoretical weight of water at that volume.

Another method is based upon the principles defined by Beer's Law, which states that the color density of a solution is directly proportional to the concentration of a test substance or coloring reagent in that solution. A solution containing 2 grams of color dye/liter would have a color density twice as intense as a solution containing 1 gram/liter of the same dye. This principle is routinely applied in laboratories whenever colorimetric analyses are performed on laboratory samples. Blood sugar, for example, is measured colorimetrically by adding appropriate reagents to serum, and then measuring the color change intensity of the serum. The color density is directly proportional to the concentration of blood sugar in the original serum sample. A kit is available to perform a calibration of this sort. The MLA Pipette Calibration Kit contains a set of special stable dye solutions of exact concentrations. These standard solutions labeled A, B, C and O are used to prepare a standard curve for the laboratory's spectrophotometer. The Kit also contains Calibration Reagent dye of known concentration and precisely measured volumes of diluent in separate Calibration Cuvettes. A pipette is calibrated by transferring (pipetting) a quantity of Calibration Reagent dye into one of the pre-measured Calibration Cuvettes. Since the dye concentration and diluent quantity are known entities, the color intensity of the final solution in the cuvette is directly proportional to the quantity of dye pipetted.

Comparison of this color intensity to the standard curve, derived from the standard solutions contained in the Kit, will yield the volume delivered by the pipette. All measurements of color intensity for both Standard Cuvettes and Calibration Cuvettes are read in absorbance units and must be made on the same spectrophotometer.

II. SUPPLIES AND EQUIPMENT

Water	OR	MLA Pipette Calibration Kit
Weigh boats		Spectrophotometer
Analytical balance (micrograms)		Cuvette

III. PROCEDURE

A. Gravimetric Method

The calibration procedure described below for using 20 replicate measurements should be performed quarterly and on new pipettes before use. For monthly checks, use the same procedure except that only 2 readings are necessary at the appropriate volumes.

All specifications apply to measurements of water at an ambient temperature range of 18 to 25^0C. Due to evaporation of water, it is imperative that there be no delays in taking the readings, especially when calibrating volumes 100 µL. Water evaporation can lead to poor coefficient of variation values due to weight-over-time-changes. Although speed is imperative, accuracy should not be sacrificed.

1. Obtain a beaker of distilled, deionized water.

2. Place a small beaker or weighing dish on a precision microgram analytical balance. Adjust the weight to zero with the tare knob.

3. Put a pipette tip on the pipette to be calibrated. Rinse the tip 2 to 3 times with water, withdraw a sample, then dispense the water into the empty container on the balance pan.

4. Record the weight (see Appendix A), reset the pan arrest, and deliver a second aliquot of water into the container on the balance pan. Record the new weight.

5. Repeat this process 20 times recording the weight each time.

6. Calculations

    a. To determine the weight of the water in the container after each pipetting, subtract the weight of the water in the container before delivery from the pipette from the weight of the water in the container after delivery from the pipette (see Appendix A). The difference will represent the weight of the water delivered by the pipette.

    b. To calculate the mean pipetting volume, add up all the numbers in column 3 (Appendix A) and divide by 20.

    c. Calculate the standard deviation (SD) of the values in column 3.

d. Calculate precision using the coefficient of variation (relative %):

$$\frac{SD \quad X \quad 100}{Mean}$$

e. Calculate accuracy (Mean Error):

Mean Volume - Volume displayed on pipette

f. Calculate % Mean Error:

$$\frac{\text{Mean Volume - Volume Displayed}}{\text{Volume Displayed}} \quad X \quad 100$$

B. Beer's Law Method

1. Carefully open a Calibration Cuvette and place it in the work station provided at the front of this Kit. SAVE THE RUBBER STOPPER. Lay the stopper upside-down on the work station taking special care not to discard any diluent that may have adhered to the stopper.

2. Using the pipette you wish to test, withdraw one sample of diluent liquid from Calibration Cuvette. Discard this sample. If testing a pipette with disposable tips, discard the tip too.

3. Open the vial of Calibration Reagent and place it in the work station next to the Calibration Cuvette.

   Use Calibration Reagent 1 for 10-50 microliter pipettes.
   Use Calibration Reagent 2 for 51-250 microliter pipettes.
   Use Calibration Reagent 3 for 251-1000 microliter pipettes.

4. Using a fresh tip, withdraw one sample of Calibration Reagent dye, wipe the outside surface of the pipette tip, and then dispense that sample into the Calibration Cuvette. Use the pipette manufacturer's recommended pipetting technique. Replace the screw cap on the Calibration Reagent Vial. Caution: DO NOT INTERCHANGE CALIBRATION REAGENT VIAL SCREW CAPS.

5. Replace the rubber stopper on the Calibration Cuvette and thoroughly mix the solution by inverting the Cuvette 10 times. CALIBRATION CUVETTE CONTENTS MUST BE THOROUGHLY MIXED TO ENSURE KIT PRECISION.

6. Repeat steps 1-5 four times for each pipette to be tested.

7. Using the "O" Standard Cuvette, re-check the zero point of the spectrophotometer (at 515 nm) and adjust accordingly. Read the absorbance value of the pipette sample Calibration Cuvette. Wipe all cuvettes carefully with a clean dry tissue before reading the spectrophotometer.

8. Record all data on the Quality Control Sheet.

9. Use a linear regression Menu to obtain delivered volumes by inputting the absorbance values. Calculate the mean, standard deviation, and coefficient of variation.

10. The coefficient of variation (CV) must be less than 3.0%. If unacceptable, report to the supervisor for further instructions.

IV. ROUTINE MAINTENANCE

In addition to routine calibration, pipettes should be cleaned and lubricated as recommended by the manufacturer.

V. PROCEDURAL NOTES

Optimum accuracy and precision occur when pipetting aqueous liquids with moderate viscosity. The "blow out" stroke is essential for accuracy and is subject to user technique. A motorized pipette may improve accuracy and precision. Positive displacement pipettes are most useful for measurement of viscous, volatile and dense liquids. Without compromising speed and efficiency, they eliminate the possibility of aerosol contamination and subsequent cross-contamination of the samples. This is especially critical in PCR methodology.

VI. RESULTS

Guidelines for accuracy and precision measurements of pipettes:

Volume (µL)	Accuracy (Relative %)	Precision (Relative %)
0.5	≤5.0	≤3.0
1	≤2.5	≤1.5
5	≤1.5	≤0.6
10-20	≤1.0	≤0.5
50-100	≤1.0	≤0.3
200-1000	≤0.8	≤0.2
2500-5000	≤0.6	≤0.2

INITIAL AND QUARTERLY PIPETTE CALIBRATION

Pipette Brand_____Pipette Number_____ Pipette volume_____

Date_____Technician_____

(1) Sample No.	(2) Balance Reading (µg)	(3) Weight Pipetted (µg)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		

Mean Pipetting Volume = Accuracy (Mean Error) = Coefficient of Variation=

Standard Deviation = % Mean Error =